

SEQUENCE LISTING

<110> C. Frank Bennett
Nicholas M. Dean
Lex M. Cowser

<120> ANTISENSE MODULATION OF INDUCIBLE NITRIC OXIDE SYNTHASE EXPRESSION

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<160> 182

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~.000 ±

20

1300 Qian et al.

12113 20

112 > DNA

3.13 Artificial Sequence

• 123> Antisense Oligonucleotide

THE JOURNAL OF CLIMATE VOL. 16, 2003

<210> 3

<211> 4145

<212> DNA

THE JOURNAL OF

<222> (207) . . . (3668)

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Phe Lys Glu Ala Lys Ile Glu Glu His Leu Ala Arg Val Glu Ala Val
155 160 165

aca aag gag ata gaa aca aca gga acc tac caa ctg acg gga gat gag 761
Thr Lys Glu Ile Glu Thr Thr Gly Thr Tyr Gln Leu Thr Gly Asp Glu
170 175 180 185

ctc atc ttc gcc acc aag cag gcc tgg cgc aat gcc cca cgc tgc att 809
Leu Ile Phe Ala Thr Lys Gln Ala Trp Arg Asn Ala Pro Arg Cys Ile
190 195 200

ggg agg atc cag tgg tcc aac ctg cag gtc ttc gat gcc cgc agc tgt 857
Gly Arg Ile Gln Trp Ser Asn Leu Gln Val Phe Asp Ala Arg Ser Cys
205 210 215

tcc act gcc cgg gaa atg ttt gaa cac atc tgc aga cac gtg cgt tac 905
Ser Thr Ala Arg Glu Met Phe Glu His Ile Cys Arg His Val Arg Tyr
220 225 230

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Ser Thr Asn Asn Gly Asn Ile Arg Ser Ala Ile Thr Val Phe Pro Gln
235 240 245

cgg agt gat ggc aag cac gac ttc cgg gtg tgg aat gct cag ctc atc 1001
Arg Ser Asp Gly Lys His Asp Phe Arg Val Trp Asn Ala Gln Leu Ile
250 255 260 265

cgc tat gct ggc tac cag atg cca gat ggc agc atc aga ggg gac cct 1049
Arg Tyr Ala Gly Tyr Gln Met Pro Asp Gly Ser Ile Arg Gly Asp Pro
270 275 280

gac aac gta gaa ttc act cag ctg tgc atc gac ctg gac tgg aag ccc 1097
Ala Asn Val Glu Phe Thr Gln Leu Cys Ile Asp Leu Gly Trp Lys Pro
285 290 295

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Arg Asp Pro Glu Leu Phe Glu Ile Pro Pro Asp Leu Val Leu Glu Val
315 320 325

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Trp Tyr Ala Leu Pro Ala Val Ala Asn Met Leu Leu Glu Val Gly Gly
350 355 360

ctg gag ttc cca ggg tgc ccc ttc aat ggc tgg tac atg ggc aca gag 1337
Leu Glu Phe Pro Gly Cys Pro Phe Asn Gly Trp Tyr Met Gly Thr Glu
365 370 375

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Ile Gly Val Arg Asp Phe Cys Asp Val Gln Arg Tyr Asn Ile Leu Glu
380 385 390

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Glu Val Gly Arg Arg Met Gly Leu Glu Thr His Lys Leu Ala Ser Leu
395 400 405

tgg aaa gac cag gct gtc gtt gag atc aac att gct gtg atc cat agt 1481
Trp Lys Asp Gln Ala Val Val Glu Ile Asn Ile Ala Val Ile His Ser
410 415 420 425

ttt cag aag cag aat gtg acc atc atg gac cac cac tcg gct gca gaa 1529
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Ser Phe Met Lys Tyr Met Gln Asn Glu Tyr Arg Ser Arg Gly Gly Cys
445 450 455

ccg gca gac tgg att tgg ctg gtc cct ccc atg tct ggg aac atc acc 1625
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460 465 470

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Pro Val Phe His Gln Glu Met Leu Asn Tyr Val Leu Ser Pro Phe Tyr

Tyr Tyr Gln Val Glu Ala Trp Lys Thr His Val Trp Gln Asp Glu Lys
490 495 500 505 1769
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510 515 520
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Arg Val Thr Ile Leu Phe Ala Thr Glu Thr Gly Lys Ser Glu Ala Leu
540 545 550 1865
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Gly Glu Lys Leu Lys Ser Leu Phe Met Leu Lys Glu Leu Asn Asn
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Lys Phe Arg Tyr Ala Val Phe Gly Leu Gly Ser Ser Met Tyr Pro Arg
620 625 630 2105
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Phe Cys Ala Phe Ala His Asp Ile Asp Gln Lys Leu Ser His Leu Gly
635 640 645 2153
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Gln Ser Glu Ser Ser Met Gly Ile Gly Asp Glu Leu Ser Gly Gln
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Glu Thr Phe Asp Val Arg Gly Lys Gln His Ile Gln Ile Pro Lys Leu			
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Tyr Thr Ser Asn Val Thr Trp Asp Pro His His Tyr Arg Leu Val Gln			
700	705	710	
gac tca cag cct ttg gac ctc agc aaa gcc ctc agc agc atg cat gcc			2393
Asp Ser Gln Pro Leu Asp Leu Ser Lys Ala Leu Ser Ser Met His Ala			
715	720	725	
aag aac gtg ttc acc atg agg ctc aaa tct cgg cag aat cta caa aqt			2441
Lys Asn Val Phe Thr Met Arg Leu Lys Ser Arg Gln Asn Leu Gln Ser			
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ccg aca tcc agc cgt gcc acc atc ctg gtg gaa ctc tcc tgt gag gat			2489
Pro Thr Ser Ser Arg Ala Thr Ile Leu Val Glu Leu Ser Cys Glu Asp			
750	755	760	
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Gly Gln Gly Leu Asn Tyr Leu Pro Gly Glu His Leu Gly Val Cys Pro			
765	770	775	
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Gly Asn Gln Pro Ala Leu Val Gln Gly Ile Leu Glu Arg Val Val Asp			
780	785	790	
ggc ccc aca ccc cac cag aca gtg cgc ctg gag gac ctg gat gag agt			2633
Gly Pro Thr Pro His Gln Thr Val Arg Leu Glu Asp Leu Asp Glu Ser			
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Gly Ser Tyr Trp Val Ser Asp Lys Arg Leu Pro Pro Cys Ser Leu Ser			
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cag gcc ctc acc tac tcc ccg gac atc acc aca ccc cca acc cag ctg			2729

Leu Leu Gln Lys Leu Ala Gln Val Ala Thr Glu Glu Pro Glu Arg Gln
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agg ctg gag gcc ctg tgc cag ccc tca gag tac agc aag tgg aag ttc 2825
Arg Leu Glu Ala Leu Cys Gln Pro Ser Glu Tyr Ser Lys Trp Lys Phe
860 865 870

acc aac agc ccc aca ttc ctg gag gtg cta gag gag ttc ccg tcc ctg 2873
Thr Asn Ser Pro Thr Phe Leu Glu Val Leu Glu Glu Phe Pro Ser Leu
875 880 885

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Arg Val Ser Ala Gly Phe Leu Leu Ser Gln Leu Pro Ile Leu Lys Pro
890 895 900 905

agg ttc tac tcc atc agc tcc cgg gat cac acg ccc acq qaq atc 2969
Arg Phe Tyr Ser Ile Ser Ser Arg Asp His Thr Pro Thr Glu Ile
910 915 920

cac ctg act gtg gcc gtg gtc acc tac cac acc gga gat ggc cag ggt 3017
His Leu Thr Val Ala Val Val Thr Tyr His Thr Gly Asp Gly Gln Gly
925 930 935

ccc ctg cac cac ggt gtc tgc agc aca tgg ctc aac agc ctg aag ccc 3065
Pro Leu His His Gly Val Cys Ser Thr Trp Leu Asn Ser Leu Lys Pro
940 945 950

caa gac cca gtg ccc tgc ttt gtg cgg aat gcc agc gcc ttc cac ctc 3113
Gln Asp Pro Val Pro Cys Phe Val Arg Asn Ala Ser Ala Phe His Leu
955 960 965

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Pro Glu Asp Pro Ser His Pro Cys Ile Leu Ile Gly Pro Gly Thr Gly
970 975 980 985

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Ile Val Pro Phe Arg Ser Phe Trp Gln Gln Arg Leu His Asp Ser Gln
990 995 1000

cac aac gga gtg cgg gga aac cgc atc acc ttg gtg ttt qgg tgc cgc 3257
Ser Thr Val Pro Asp Val Gly Asp Ser Ser Thr Val Phe Gly Cys Asp

Arg Pro Asp Glu Asp His Ile Tyr Gln Glu Glu Met Leu Glu Met Ala
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cag aag ggg gtg ctg cat gcg gtg cac aca gcc tat tcc cgc ctg cct 3353
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Gly Lys Pro Lys Val Tyr Val Gln Asp Ile Leu Arg Gln Gln Leu Ala
1050 1055 1060 1065

agc gag gtg ctc cgt gtg ctc cac aag gag cca ggc cac ctc tat gtt 3449
Ser Glu Val Leu Arg Val Leu His Lys Glu Pro Gly His Leu Tyr Val
1070 1075 1080

tgc ggg gat gtg cgc atg gcc cgg gac gtg gcc cac acc ctg aag cag 3497
Cys Gly Asp Val Arg Met Ala Arg Asp Val Ala His Thr Leu Lys Gln
1085 1090 1095

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Leu Val Ala Ala Lys Leu Lys Leu Asn Glu Glu Gln Val Glu Asp Tyr
1100 1105 1110

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Phe Phe Gln Leu Lys Ser Gln Lys Arg Tyr His Glu Asp Ile Phe Gly
1115 1120 1125

gct gta ttt cct tac gag gcg aag aag gac agg gtg gcg gtg cag ccc 3641
Ala Val Phe Pro Tyr Glu Ala Lys Lys Asp Arg Val Ala Val Gln Pro
1130 1135 1140 1145

agc agc ctg gag atg tca gcg ctc tga gggcctacag gagggttaa 3688
Ser Ser Leu Glu Met Ser Ala Leu
1150

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tggggagatg gaggaaagtg atatccccca gcctcaagtc ttatttcctc aacgttgctc 3808

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gctatcgac cactgtgtat ttaactgcct tgtgtacagt tatttatgcc tctgtattta 4048

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Met Ala Cys Pro Trp Lys Phe Leu Phe
1 5
aag acc aaa ttc cac cag tat gca atg aat ggg gaa aaa gac atc aac 281
Lys Thr Lys Phe His Gln Tyr Ala Met Asn Gly Glu Lys Asp Ile Asn
10 15 20 25
aac aat gtg gag aaa gcc ccc tgt gcc acc tcc agt cca gtg aca cag 329
Asn Asn Val Glu Lys Ala Pro Cys Ala Thr Ser Ser Pro Val Thr Gln
30 35 40
gat gac ctt cag tat cac aac ctc agc aag cag cag aat gag tcc ccg 377
Asp Asp Ile Gln Tyr His Asn Leu Ser Lys Gln Gln Asn Glu Ser Pro
45 50 55
cag ccc ctc gtg gag acg gga aag aag tct cca gaa tct ctg gtc aag 425
Gln Pro Leu Val Glu Thr Gly Lys Lys Ser Pro Glu Ser Leu Val Lys
60 65 70
ctg gat gca acc cca ttg tcc tcc cca cgg cat gtg agg atc aaa aac 473
Leu Asp Ala Thr Pro Leu Ser Ser Pro Arg His Val Arg Ile Lys Asn
75 80 85
tgg ggc agc ggg atg act ttc caa gac aca ctt cac cat aag gcc aaa 521
Trp Gly Ser Gly Met Thr Phe Gln Asp Thr Leu His His Lys Ala Lys
90 95 100 105
ggg att tta act tgc agg tcc aaa tct tgc ctg ggg tcc att atg act 569
Gly Ile Leu Thr Cys Arg Ser Lys Ser Cys Leu Gly Ser Ile Met Thr
110 115 120
ccc aaa agt ttc acc aaa gaa ccc agg gac aag cct acc cct cca dat 617
Pro Lys Ser Leu Thr Arg Gly Pro Arg Asp Lys Pro Thr Pro Pro Asp
125 130 135
gag ctt cta cct caa gct atc gaa ttt gtc aac caa tat tac ggc tcc 665
Glu Leu Leu Pro Gln Ala Ile Glu Phe Val Asn Gln Tyr Tyr Gly Ser
140 145 150

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Thr Lys Glu Ile Glu Thr Thr Gly Thr Tyr Gln Leu Thr Gly Asp Glu			
170	175	180	185
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Leu Ile Phe Ala Thr Lys Gln Ala Trp Arg Asn Ala Pro Arg Cys Ile			
190	195	200	
ggg agg atc cag tgg tcc aac ctg cag gtc ttc gat gcc cgc agc tgt			857
Gly Arg Ile Gln Trp Ser Asn Leu Gln Val Phe Asp Ala Arg Ser Cys			
205	210	215	
tcc act gcc cgg gaa atg ttt gaa cac atc tgc aga cac gtg cgt tac			905
Ser Thr Ala Arg Glu Met Phe Glu His Ile Cys Arg His Val Arg Tyr			
220	225	230	
tcc acc aac aat ggc aac atc agg tcg gcc atc acc gtg ttc ccc cag			953
Ser Thr Asn Asn Gly Asn Ile Arg Ser Ala Ile Thr Val Phe Pro Gln			
235	240	245	
cgg agt gat ggc aag cac gac ttc cgg gtg tgg aat gct cag ctc atc			1001
Arg Ser Asp Gly Lys His Asp Phe Arg Val Trp Asn Ala Gln Leu Ile			
250	255	260	265
cgc tat gct ggc tac cag atg cca gat ggc agc atc aga ggg gac cct			1049
Arg Tyr Ala Gly Tyr Gln Met Pro Asp Gly Ser Ile Arg Gly Asp Pro			
270	275	280	
gcc aac gtg gaa ttc act cag ctg tgc atc gac ctg ggc tgg aag ccc			1097
Ala Asn Val Glu Phe Thr Gln Leu Cys Ile Asp Leu Gly Trp Lys Pro			
285	290	295	
aag tac ggc cgc ttc gat gtg gtc ccc ctg gtc ctg cag gcc aat gac			1145
Lys Tyr Gly Arg Phe Asp Val Val Pro Leu Val Leu Gln Ala Asn Gly			
300	305	310	
cgt gac cct gag ctc ttc gaa atc cca cct gac ctt gtg ctt gag gtg			1193
Arg Asp Pro Glu Leu Phe Glu Ile Pro Pro Asp Leu Val Leu Glu Val			
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330	335	340	345	
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ctg gag ttc cca ggg tgc ccc ttc aat ggc tgg tac atg ggc aca gag Leu Glu Phe Pro Gly Cys Pro Phe Asn Gly Trp Tyr Met Gly Thr Glu				1337
365	370	375		
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380	385	390		
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395	400	405		
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430	435	440		
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445	450	455		
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460	465	470		
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475	480	485		
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490	495	500	505	

510	515	520	
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525	530	535	
aga gtc acc atc ctc ttt gcg aca gag aca gga aaa tca gag gcg ctg Arg Val Thr Ile Leu Phe Ala Thr Glu Thr Gly Lys Ser Glu Ala Leu			1865
540	545	550	
gcc tgg gac ctg ggg gcc tta ttc agc tgt gcc ttc aac ccc aag gtt Ala Trp Asp Leu Gly Ala Leu Phe Ser Cys Ala Phe Asn Pro Lys Val			1913
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ctg ttg gtg acc agt acg ttt ggc aat gga gac tgc cct ggc aat Leu Leu Val Val Thr Ser Thr Phe Gly Asn Gly Asp Cys Pro Gly Asn			2009
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620	625	630	
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635	640	645	
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650	655	660	665
gag gac gcc ttc cgc aca tgg gcc gtg caa acc ttc aag gca gcc tgt Glu Asp Ala Phe Arg Ser Thr Ala Val Gln Thr Phe Lys Ala Ala Cys			2249
670	675	680	

685	690	695	
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715	720	725	
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730	735	740	745
ccg aca tcc agc cgt gcc acc atc ctg gtg gaa ctc tcc tgt gag gat Pro Thr Ser Ser Arg Ala Thr Ile Leu Val Glu Leu Ser Cys Glu Asp			2489
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765	770	775	
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780	785	790	
ggc ccc aca ccc cac cag aca gtg cgc ctg gag gac ctg gat gag agt Gly Pro Thr Pro His Gln Thr Val Arg Leu Glu Asp Leu Asp Glu Ser			2633
795	800	805	
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810	815	820	825
cag gcc ctc aac tac tcc ccg dac atc acc aca ccc cca acc cag ctg Gln Ala Leu Thr Tyr Ser Pro Asp Ile Thr Pro Pro Thr Gln Leu			2729
830	835	840	
ctg ctc caa aag ctg gcc cag gtg gcc aca gaa gag cct gag aga cag Leu Leu Gln Lys Leu Ala Gln Val Ala Thr Glu Glu Pro Glu Arg Gln			2777
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4145

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<13> Artificial Sequence

<23> PCR Primer

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23

<210> 12
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22

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<223> PCR Probe

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27

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<212> DNA

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5

54

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Trp Val Trp Val Ser Leu Leu Val Ala Ala Gly Thr Val Gln Pro Ser

102

Asp Ser Gln Ser Val Cys Ala Gly Thr Glu Asn Lys Leu Ser Ser Leu			
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tct gac ctg gaa cag cag tac cga gcc ttg cgc aag tac tat gaa aac			193
Ser Asp Leu Glu Gln Gln Tyr Arg Ala Leu Arg Lys Tyr Tyr Glu Asn			
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Cys Glu Val Val Met Gly Asn Leu Glu Ile Thr Ser Ile Glu His Asn			
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cgx gac ctc tcc ttc ctg cgg tct gtt cga gaa gtc aca ggc tac gtg			294
Arg Asp Leu Ser Phe Leu Arg Ser Val Arg Glu Val Thr Gly Tyr Val			
75	80	85	
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Leu Val Ala Leu Asn Gln Phe Arg Tyr Leu Pro Leu Glu Asn Leu Arg			
90	95	100	
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Ile Ile Arg Gly Thr Lys Leu Tyr Glu Asp Arg Tyr Ala Leu Ala Ile			
105	110	115	
ttt tta aac tac aga aaa gat gga aac ttt gga ctt caa gaa ctt gga			438
Phe Leu Asn Tyr Arg Lys Asp Gly Asn Phe Gly Leu Gln Glu Leu Gly			
120	125	130	135
tta aag aac ttg aca gaa atc cta aat ggt gga gtc tat gta gac cag			486
Leu Lys Asn Leu Thr Glu Ile Leu Asn Gly Gly Val Tyr Val Asp Gln			
140	145	150	
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Asn Lys Phe Leu Cys Tyr Ala Asp Thr Ile His Trp Gln Asp Ile Val			
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Arg Asn Pro Trp Pro Ser Asn Leu Thr Leu Val Ser Thr Asn Gly Ser			
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Gln Thr Phe Val Tyr Asn Pro Thr Thr Phe Gln Leu Glu His Asn Phe
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Gly Asn Ile Tyr Ile Thr Asp Asn Ser Asn Leu Cys Tyr Tyr His Thr
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Lys Ser His Gln Asn Gly Ser Pro Gln Leu Leu Thr Gly Thr Ala Gln
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Asn Val Pro Glu Ser Leu Asp Lys Leu His Val Thr Ser Thr Arg Pro
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Asp Lys Pro Thr Pro Leu Glu Glu Leu Pro His Ala Ile Glu Phe
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Gln Arg Tyr Asn Ile Leu Glu Glu Val Gly Arg Arg Met Gly Leu Glu					
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Asp His His Thr Ala Ser Glu Ser Phe Met Lys His Met Gln Asn Glu					
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Tyr Arg Ala Arg Gly Gly Cys Pro Ala Asp Trp Ile Trp Leu Val Pro					
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Pro Val Ser Gly Ser Ile Thr Pro Val Phe His Gln Glu Met Leu Asn					
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Tyr Val Leu Ser Pro Phe Tyr Tyr Gln Ile Glu Pro Trp Lys Thr					
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His Ile Trp Gln Asn Glu Lys Leu Arg Pro Arg Arg Glu Ile Arg					
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cag ctc cct atc ttg aag ccc cgc tac tac tcc atc agc tcc tcc cag	2979		
Gln Leu Pro Ile Leu Lys Pro Arg Tyr Tyr Ser Ile Ser Ser Ser Gln			
895	900	905	
gac cac acc ccc tcg gag gtt cac ctc act gtg gcc gtg gtc acc tac	3027		
Asp His Thr Pro Ser Glu Val His Leu Thr Val Ala Val Val Thr Tyr			
910	915	920	
cgc acc cga gat ggt cag ggt ccc ctg cac cat ggt gtc tgc agc act	3075		
Arg Thr Arg Asp Gly Gln Gly Pro Leu His His Gly Val Cys Ser Thr			
925	930	935	940
tgg atc agg aac ctg aag ccc cag gac cca gtg ccc tgc ttt gtg cga	3123		
Trp Ile Arg Asn Leu Lys Pro Gln Asp Pro Val Pro Cys Phe Val Arg			
945	950	955	
agt gtc agt ggc ttc cag ctc cct gag gac ccc tcc cag cct tgc atc	3171		
Ser Val Ser Gly Phe Gln Leu Pro Glu Asp Pro Ser Gln Pro Cys Ile			
960	965	970	
ctc att ggg cct ggt acg ggc att gct ccc ttc cga agt ttc tgg cag	3219		
Leu Ile Gly Pro Gly Thr Gly Ile Ala Pro Phe Arg Ser Phe Trp Gln			
975	980	985	
cag cgg ctc cat gac tcc cag cac aaa ggg ctc aaa gga ggc cgc atg	3267		
Gln Arg Leu His Asp Ser Gln His Lys Gly Leu Lys Gly Gly Arg Met			
990	995	1000	
agc ttg gtg ttt ggg tgc cgg cac ccg gag gag gac cac ctc tat cag	3315		
Ser Leu Val Phe Gly Cys Arg His Pro Glu Glu Asp His Leu Tyr Gln			
1005	1010	1015	1020
gaa gaa atg cag qaq atg gtc cgc aag aqa gtg ctg ttc cag gtg cac	3363		
Glu Glu Met Gln Glu Met Val Arg Lys Arg Val Leu Phe Gln Val His			
1025	1030	1035	
aca ggc tac tcc cgg ctg ccc ggc aaa ccc aag gtc tac gtt cag gac	3411		
Thr Gly Tyr Ser Arg Leu Pro Gly Lys Pro Lys Val Tyr Val Gln Asp			
1040	1045	1050	
.....	3459

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Glu Gln Gly His Leu Tyr Ile Cys Gly Asp Val Arg Met Ala Arg Asp
1070 1075 1080

gtg gct acc aca ttg aag aag ctg gtg gcc acc aag ctg aac ttg agc 3555
Val Ala Thr Thr Leu Lys Lys Leu Val Ala Thr Lys Leu Asn Leu Ser
1085 1090 1095 1100

gag gag cag gtg gaa gac tat ttc ttc cag ctc aag agc cag aaa cgt 3603
Glu Glu Gln Val Glu Asp Tyr Phe Phe Gln Leu Lys Ser Gln Lys Arg
1105 1110 1115

tat cat gaa gat atc ttc ggt gca gtc ttt tcc tat ggg gca aaa aag 3651
Tyr His Glu Asp Ile Phe Gly Ala Val Phe Ser Tyr Gly Ala Lys Lys
1120 1125 1130

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400-112
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• 25+ 116
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